Environmental Health Perspectives
Vol. 72, pp. 203-210, 1987

Teratological Research Using *In Vitro* Systems. I. Mammalian Whole Embryo Culture

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Approximately 390 literature references (through spring 1986) were reviewed for mammalian whole embryo culture procedures, with particular attention to the development of those cultures as systems for teratogenicity testing. The existing procedures could be conveniently divided into three groups, which are defined by the periods of embryogenesis that they embrace: preimplantation, peri-implantation, and post-implantation culture systems. The literature on peri-implantation embryo culture was sparse, and it did not appear that this procedure is being actively developed as a teratogen screening test. The extensive literature on both preimplantation and postimplantation embryo culture suggested considerable use of these two methods in evaluating embryotoxicants.

The following discussion was compiled from information gleaned from all references. However, in the interest of brevity, only representative articles are specifically cited. Because the background and methodology for each system are distinct, each system will be discussed separately.

Preimplantation Embryos

Introduction

The procedures for the culture of preimplantation mouse embryos from the two-cell to the blastocyst stage were developed in the late 1950s and early 1960s, primarily through the work of Whitten and Brinster (*1*). Kane (*2*) described conditions for culturing preimplantation rabbit embryos in a defined medium. Some success has been achieved in culturing preimplantation embryos from larger species, including human, but these species require undefined media that contain serum.

Interest in preimplantation embryo culture as a screen for environmental teratogenesis arose from observations that preimplantation embryos, both *in vivo* and *in vitro*, are highly susceptible to the damaging effects of ionizing radiation. Simple procedures are available for obtaining metaphase chromosomal spreads from preimplantation embryos, thus making them ideal for studies on agents for which chromosomal damage is suspected of being the mechanism of reproductive toxicity. A literature search through spring 1986 revealed 53 references in which preimplantation embryos were used to evaluate the embryotoxicity of 48 different environmental agents. Preimplantation embryos from rats were used in one of these studies, and the remainder used embryos from mice.

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Methodology

Both general methods of preimplantation mouse embryo culture (*1*) and methods applicable to teratology studies (*3*) have been reviewed and described. Female mice are both time-ovulated and superovulated with gonadotropins, mated to fertile males, and examined for vaginal plugs to confirm positive mating. Pregnant females are killed 36 hr after ovulation, the oviducts are excised, and two-cell stage embryos are flushed from the oviducts. Because of the hormonal superovulation, 20 to 40 embryos can be obtained from each female. The embryos are transferred to a chemically defined medium and incubated at 37°C in an atmosphere of 5% carbon dioxide in air. Cultured embryos develop normally for 72 hr up to the blastocyst stage. At this point, they may be examined directly, transferred to outgrowth medium for an additional 120 hr, and then examined for development of embryonic germinal tissue, or transferred to pseudopregnant females and examined at various times throughout gestation for normal embryonic development.

Critical Review

Forty-eight different agents (including chemicals, physical agents such as X-irradiation, and biological agents such as viruses and antibodies) have been used on cultured preimplantation embryos to evaluate embryotoxicity. The basic culture procedures appeared to be uniform among different laboratories, with the major variations being the choice of medium and strain of

mouse. Specific aspects of a system that relate to its use as a teratology screen (e.g., end points, presence of metabolic activation) are much more variable.

End Points. The major end points used are the survival of embryos and the visual appearance of surviving embryos. Survival provides only a quantal response (i.e., dead or alive). Questions can also be raised as to whether a dead embryo represents a true embryotoxicity or merely a generalized cytotoxic response.

The appearance of surviving embryos is used to evaluate the ability of the test agent to inhibit normal growth and morphogenesis of the preimplantation embryos. However, it is not usually determined whether a test embryo at an earlier developmental stage than control embryos has experienced developmental retardation or has died at the early stage and not yet noticeably degenerated. Another problem arises when authors report no effect of an agent based on the ability of treated embryos to achieve a "normal" morphology for the developmental stage at which they are examined (e.g., morula, blastocyst, etc.). It has been demonstrated (*3*) that embryos that have a normal appearance at the gross level may not be normal when examined by other means. The major mechanism for this misjudgment appears to be selective cell death, whereby enough cells remain to give the embryo an overtly normal appearance. Many authors supplement the gross examination with a determination of the actual number of cells in the treated and control embryos. Although this procedure will identify embryos with a significantly reduced cell number, it will not identify embryos in which a small number of cells killed by the test agent is critical to the further development of the embryo.

This latter point has been evaluated by examining further development of treated embryos in two ways. First, embryos cultured up to the blastocyst stage can be transferred to outgrowth medium (*4*). This medium allows blastocysts to undergo a further pseudodevelopment. Normal outgrowths will form two distinct anlage consisting of trophoblast cells, which are representative of extraembryonic structures, and an inner cell mass (ICM), which gives rise to the embryo proper. The ICM will further differentiate into embryonic ectoderm and endoderm. Agents have been described (*5*) that apparently selectively destroy the relatively few cells in the blastocyst that give rise to the ICM. Thus, apparently normal blastocysts yield outgrowths composed entirely of trophoblast cells.

The second means used to evaluate postimplantation developmental potential after *in vitro* preimplantation exposure to agents is embryo transfer (*6*). In this procedure, cultured blastocysts are transferred to the uterus of pseudopregnant females. The females can be killed and the embryos examined for abnormal development at any time during gestation. One limitation of this procedure is the relatively low (about 50%) success rate of development of all transferred embryos, including untreated controls.

Some end points provide more quantitative data than gross morphological appearance. Cytological parameters, such as the number of micronuclei, the nuclear labeling index (*7*), and the rate of sister chromatid exchange, (*8*) have been determined in control versus treated cultured embryos. Biochemical indexes, such as rate of incorporation of ^{35}S-methionine into protein (*9*) and incorporation of ^{3}H-uridine into RNA (*10*), have also been used. These quantitative end points also provide information about possible mechanisms of action of embryotoxic agents. The cytological parameters reveal agents that act through genetic mechanisms such as mutation; the biochemical parameters reveal agents that act through disruption of normal cellular processes such as RNA or protein synthesis.

Dose Response. In most of the studies in which chemical agents were used as a toxic insult, the embryos were placed directly into medium containing the chemical, and the embryo was exposed to the chemical throughout the culture period (24–72 hr). Only three studies were found in which embryos were exposed for short periods of time and then transferred to fresh medium (*11–13*). Exposure to physical insults such as ultraviolet or X-irradiation was always on a short-term basis (*14*). In general, when multiple levels of a test agent were used, a dose-response was obtained for at least one of the end points examined by the authors. In many cases, the authors provided a graphical depiction, but no formal statistical analysis of the dose-response (e.g., regression analysis, analysis of variance, etc.). It was also apparent that most of the agents and end points used produced very steep dose-response curves. The concentrations causing 0% and 100% effects often lay within an order of magnitude of each other.

The lowest concentrations of chemical agents reported by most authors as producing a significant response were in the micromolar range. The most potent agent studied with the preimplantation embryo system was Shigella toxin, which inhibited protein synthesis and further development of embryos at concentrations in the medium as low as 0.1 pg/mL (*15*). The least toxic agent tested was phenol, which yielded observable effects only at concentrations in the medium above 5 mM (470 μg/mL). Several agents were reported that had no observable effects on *in vitro* embryonic development at the highest concentrations used.

Types of Compounds That Can Be Studied. The 48 agents that have been used in the preimplantation embryo culture system to evaluate embryotoxicity are representative of a number of classes. These include, but are not limited to, various forms of radiation, heavy metals, chemotherapeutic agents, nonsteroidal anti-inflammatory drugs, viruses, plant and microbial toxins, and specific enzyme and metabolic inhibitors. The vast majority of chemical agents used were water-soluble compounds that were readily soluble in the culture medium. Addition of human serum to the culture medium can also be used to identify some embryotoxic serum factors (*16*).

The standard protocol for preimplantation embryo culture calls for injecting drops of medium under paraffin oil in culture dishes. The overlaid paraffin oil allows

gas diffusion, but retards evaporation of the medium. This paraffin oil extracts lipophilic compounds from the medium (*17*). Thus, the study of lipophilic agents requires the use of nonstandard culture procedures. Methods used to incorporate poorly soluble compounds into the medium included direct weighing of prostaglandin PGF_{2a} into the medium during preparation (*18*); and dissolution of phorbol esters (*19*) and benzo[a]pyrene (*11*) in dimethylsulfoxide (DMSO), followed by addition of DMSO to the medium at final DMSO concentrations of up to 0.1%.

Metabolic Activation. Preimplantation embryos from some strains of mice have been reported to possess inducible metabolic activity toward polycyclic aromatic hydrocarbons (*20*). They have not yet been shown to possess any other xenobiotic-metabolizing capabilities. Pedersen et al. (*11*) introduced more substantial *in vitro* metabolic capabilities by incubating embryos for 30 min in medium containing benzo[a]pyrene and a standard S-9 preparation with cofactors, and then returning the embryos to conventional culture medium for further development. Burki and Sheridan (*21*) used *in vivo* metabolic activation by injecting the alkylating agent TEM into male mice before mating. Embryos fertilized by these males were then cultured and examined for chromosomal anomalies.

Time, Personnel, and Cost Requirements. Since there is no single standardized protocol for developmental toxicity testing with preimplantation embryos, time, personnel, and cost requirements vary according to the final procedures selected. For example, the number of embryos obtained per mouse varies from 6 to 12 to 30 to 40, depending on whether or not gonadotropins were used to induce superovulation. If gonadotropins were used, the increased number of embryos involved will necessitate either an increase in personnel or a decrease in number of animals used per experimental run. The choice among these options will also influence costs, since personnel costs would normally be higher than the costs of purchasing mice. However, the savings achieved by reducing personnel may be offset by an increase in the number of experimental runs needed to obtain enough embryos for statistical analysis, with a subsequent increase in total time required.

The choice of an end point will also greatly influence total time, personnel, and cost requirements. A standard preimplantation culture from initiation of timed ovulation through removal of two-cell stage embryos to the blastocyst stage takes a total of 8 days. An additional 5 days (or a total of 13 days) are needed if the blastocysts are left to develop as explants; an additional 14 to 15 days (or a total of 22–23 days) are required if the blastocysts are transferred to pseudopregnant females and allowed to develop to term. End points other than morphological development, e.g., biochemical or cytogenetic assays, will extend these times depending on the complexity of the assay and the total number of embryos to be assayed. The number of embryos used varies greatly from study to study, but a minimum of about 25 per treatment group with an average of about 100 per group seems most common.

A great deal of technical skill is required for all procedures in preimplantation mammalian embryology. Because of the small size of embryos at this stage, all manipulations must be done under a microscope. Standard culture procedures, such as sterility, must be maintained throughout. If embryo transfer techniques are used, considerable skill in surgery on mice is necessary.

Nonpersonnel costs of preimplantation embryo culture are relatively modest (T. Flynn, personal observation). Major equipment required includes dissecting microscopes, a carbon dioxide incubator, and an autoclave. Although embryo culture medium is available commercially, it can be prepared easily from inexpensive components.

Utility in Mechanistic Studies. Because of their rapid rate of cell division and the ease with which metaphase spreads can be prepared, preimplantation embryos are useful for uncovering genotoxic mechanisms of developmental toxicants. Preimplantation procedures can be extended to treatment periods before mating to evaluate reproductive hazards stemming from genotoxic effects on either male or female gametes (*22*). However, because of the limited period of gestation represented by these embryos and the limited amount of differentiation they undergo, preimplantation embryos are probably not of much value in elucidating nongenetic mechanisms of embryotoxicity.

Utility as a Screen for Teratogens. There do not appear to have been any serious efforts made toward standardizing or validating preimplantation embryo culture as a teratogen screening assay.

Peri-implantation Embryos

Introduction

A number of procedures are available for the culture of mammalian embryos at the peri-implantation stage. Wiley and Pedersen (*23*) successfully cultured mouse blastocysts to the egg cylinder stage. Rizzino and Sherman (*24*) later described a serum-free medium for mouse blastocyst culture. Tam and Snow (*25*) were able to get 60% of early primitive-streak mouse embryos to develop for 48 hr in culture. Chen and Hsu (*26*) cultured mouse embryos from the blastocyst to the limb bud stage, but the culture procedure used was complex and the success rate was low.

Procedures have also been described for the culture of peri-implantation rabbit (*27*) and rat (*28*) embryos. However, the literature on these species is not as extensive as that for the mouse.

Methodology

Apparently there is no standard methodology for culture of peri-implantation embryos. Both static and rotating culture systems have been used. Culture media have been described that are either serum-free or con-

tain one or more of the following sera: fetal calf serum, newborn calf serum, human cord serum, mouse serum, or rat serum. Embryos are cultured either in the same medium throughout or transferred to different media as culture progresses. More detailed descriptions of the individual procedures can be found in references (*23–28*).

Critical Review

Peri-implantation embryo culture does not appear to have been seriously considered as a procedure for evaluating embryotoxicity. In the literature search, only eight studies were found in which peri-implantation embryos were exposed to toxic agents. Of these eight studies, only three indicated that evaluation of mechanisms of teratogenicity was the primary goal of the study (*29–31*). Lack of standardized procedures and poor survival rate have probably kept peri-implantation embryo culture from being used more for screening teratogens.

Postimplantation Embryos

Introduction

Although methods for culturing postimplantation mammalian embryos were first described in the 1930s, New and his coworkers (*32*) are generally credited with establishing the contemporary methodology for organogenesis-stage mammalian embryo culture during the 1960s and 1970s.

Use of postimplantation embryo cultures as a teratology screen developed slowly because initial culture procedures resulted in a poor overall rate of survival of the embryos and significant differences between surviving cultured embryos and their counterparts *in vivo*. New et al. (*33*) reported on culture conditions that allowed more than 95% of rat embryos explanted from the uterus on gestation day 9 (headfold stage) to survive in culture for 48 hr. These same authors (*34*) also demonstrated that their embryonic development *in vitro* was indistinguishable from that of paired littermate controls left to develop *in utero*. Wee et al. (*35*), using New's technique, sustained day 14.75 mouse embryos in culture for limited periods to study palatal shelf rotation. Sadler (*36*) modified New's culture conditions for rat embryos and successfully cultured mouse embryos for 48 hr starting on gestation day 8 (early somite stage). Tarlatzis et al. (*37*) described a modified apparatus that allows continuous culture of rat embryos for 96 hr. Attempts have been made to culture organogenesis-stage embryos from other species, but thus far none of these species has matched the mouse or rat in either ease of culture or consistency of results.

The period of successful culture, embryonic days 9 to 11 in the rat and days 8 to 10 in the mouse, corresponds to the period of major organogenesis in these rodents. This same period is the one which, as whole animal teratology studies indicate, is most sensitive to teratological insult. Since the mid-1970s, several research groups have used whole embryo culture as a means of evaluating or elucidating mechanisms of action of known or suspect embryotoxic agents. A search of literature dating through spring 1986 revealed more than 200 studies in which postimplantation embryo culture was used to evaluate teratogens.

Methodology

The basic culture procedures derive from those described by New et al. (*33,34*). Rat embryos are explanted from the uterus at the headfold stage (gestation day 9.5), with the yolk sac and ectoplacental cone left intact. Embryos are transferred to 30 mL culture bottles, one or two embryos per bottle. The culture medium is 4 mL of whole rat serum, which must be prepared by centrifuging freshly drawn rat blood before it clots. The serum must also be heat-inactivated at 56°C for 30 min. The culture bottles are then equilibrated with a gas mixture of 5% oxygen, 5% carbon dioxide, and 90% nitrogen, sealed tightly, and rotated in a horizontal position at 40 to 50 rpm. The culture bottles are re-equilibrated at 24 and 32 hr with gas mixtures containing, respectively, 20% and 40% oxygen, with carbon dioxide remaining constant at 5% and nitrogen providing the balance.

Critical Review

A major drawback to whole embryo culture as a teratology screen is the lack of uniformity in experimental conditions between laboratories. These differences include variations in actual culture procedures, quantitation of end points, inclusion of metabolic-activating capability, and choice of species or strain.

End Points. Most authors use some criteria for evaluating the viability of the cultured embryos, with nonviable embryos being eliminated from the final data analysis. The factors used for evaluating viability include lack of obvious physical trauma (e.g., tears in the yolk sac), a regularly beating heart, and presence of an active yolk sac blood circulation.

The most common criterion used as an end point is the overall morphology of treated versus control embryos. Embryos are observed under a dissecting microscope for delayed or abnormal development overall or in specific organ systems. Various measurements such as crown-rump and head lengths can be obtained with an eyepiece reticle, and the number of dorsal somite pairs can be counted. Brown and Fabro (*38*) have described a detailed anatomical scoring system which provides a quaqntitative assessment of overall anatomical development of somite-stage rat embryos.

Biochemical end points have been used frequently to assess viability. The most common end points are the total DNA and protein contents of the embryos. Other biochemical parameters that have been measured include total RNA content; total hemoglobin content; embryonic and yolk sac DNA, RNA, and protein synthesis;

yolk sac ornithine decarboxylase activity; glycosaminoglycan staining; and lactic acid release.

Allen et al. (*39*) described a cytogenetic end point whereby the number of sister chromatid exchanges was determined in embryos exposed either *in vivo* or *in vitro* to cyclophosphamide or its metabolites.

Dose Response. Most studies used multiple doses of test agents, and in all cases in which a positive effect of the agent was observed, the effect was reported to be dose-related.

Most agents appeared to affect cultured embryos in the millimolar concentration range. Actinomycin D (*40*) was apparently the most potent substance tested; it produced significant effects in cultured embryos at a concentration in the medium of 0.3 ng/mL. Ethanol (*41*) seemed to be the least active compound tested, since significant effects were observed only at medium concentrations above 2 mg/mL. L-Glucose was ineffective at concentrations as high as 15 mg/mL (*42*). Thus, the whole embryo culture system can respond to agents in the medium over a concentration range of about 10^8.

Warner et al. (*43*) have shown that the whole embryo system can be manipulated to more closely approximate the expected *in vivo* exposure conditions. These authors determined the peak plasma concentration and half-life of an *in vivo* teratogenic dose of hydroxyurea. They duplicated these exposure conditions for cultured mouse embryos. The spectrum of abnormalities observed *in vitro* closely mimicked those seen *in vivo*.

Types of Compounds That Can Be Studied. There do not appear to be any limitations on the types of compounds or agents that can be tested with the postimplantation embryo culture system. A list compiled from literature references showed that 181 agents have been tested on rat embryos and 26 agents on mouse embryos. Because most of the chemicals used were water soluble, they were added to the medium in aqueous solution.

Embryos were exposed to water-insoluble compounds in a number of ways. Tunicamycin was administered locally to cultured embryos by first coating it onto a human eyelash and then implanting the eyelash in the region of the embryonic nasal placode (*44*). Retinoic acid (*45*) and chlorambucil (*46*) have been added to culture serum dissolved in ethanol. DMSO has been used as a solvent for several compounds such as 2-acetylaminofluorene (*47*). Sim et al. (*48*) were able to disperse jervine in an aqueous suspension by sonication. Satish et al. (*49*), using microinjection, directly injected phosphoramide mustard into various regions of cultured embryos.

Kitchin and Ebron (*50*) have made a comprehensive study on solvent toxicity and water-insoluble compound delivery to cultured embryos. These authors found that Tween 80, acetone, ethanol, and DMSO were all toxic at medium concentrations greater than 0.1%. However, corn oil could be suspended in serum by ultrasound, and it was nontoxic at concentrations up to 2.5%.

Metabolic Activation. Somite-stage mouse embryos have been shown to metabolize benzo[a]pyrene to active metabolites (*51*) and to possess an inducible cytochrome P-450 activity for 2-acetylaminofluorene metabolism (*52*). Day 10 rat embryos possess a cytochrome P-450 activity that can be induced transplacentally with 3-methylcholanthrene (*53*), as well as an endogenous activity capable of metabolizing aflatoxin (*54*). Several approaches have been taken to incorporate metabolic activation capabilities into the postimplantation embryo culture system.

Fantel et al. (*55*) first reported on the inclusion of an S-9 system, which was comparable to that used in *in vitro* mutagenicity assays, into the embryo culture medium. These authors showed that cyclophosphamide, a toxic compound known to require metabolic activation, was toxic to cultured embryos only when the S-9 fraction and appropriate cofactors were incorporated into the culture system. Kitchin et al. (*56*) modified this approach slightly by using a purified microsomal fraction in place of the crude S-9 preparation.

Oglesby et al. (*57*) reported on the successful co-culture of rat, hamster, and rabbit hepatocytes with rat embryos. This system provides a means of examining species differences in sensitivity to chemical teratogens.

In cases where the metabolites of a suspect teratogen are known and chemically stable, they can be added directly to the culture medium. This approach provides information on the nature of the proximate teratogen. Mirkes et al. (*58*) studied the effects of three stable metabolites of cyclophosphamide on cultured embryos. These authors showed that only one metabolite, phosphoramide mustard, could duplicate the effects seen with bioactivated cyclophosphamide. Mirkes and other research groups have used this approach on other parent compounds and their stable metabolites.

Various methods have been used for obtaining *in vivo* bioactivation of test compounds, including direct *in vivo* exposure. Beaudoin and Fisher (*59*) administered a number of environmental toxins to pregnant rats either 4 or 24 hr before recovery and culture of day-10 rat embryos. All compounds tested were shown to inhibit *in vitro* development of the embryos.

An alternative approach to obtaining *in vivo* bioactivation is the use of serum from animals treated with the test compound as the culture medium. Klein et al. (*60*) treated adult rats with either cadmium or cyclophosphamide and then obtained serum at various intervals after treatment. The response of cultured embryos to the various sera was dependent on the time interval between treatment of the animal and withdrawal of the blood. Sadler (*61*) showed that serum obtained from diabetic rats induced abnormalities in cultured mouse embryos. Two hypolipidemic drugs, which were known *in vivo* teratogens, were shown to act directly on cultured embryos (*62*), while hypolipidemic serum from treated animals did not induce anomalies. This finding suggested that the drugs themselves and not maternal hypolipemia were the cause of *in vivo* teratogenesis.

Klein and his co-workers have extended the *in vivo* bioactivation approach by successfully culturing rat embryos on human (*63*) and monkey (*64*) serum supple-

mented with glucose. It was shown in the former study that serum from humans undergoing treatment with either chemotherapeutic or anticonvulsant agents was teratogenic to embryos *in vitro*.

Time, Personnel, and Cost Requirements. Sadler and Warner (*65*) have evaluated the time, material, and costs for screening one compound using postimplantation mouse embryo culture. Their analysis was based on using four groups of 21 embryos, or 84 total embryos. The total time needed was estimated at 40 technician-hours; thus, the overall time required depends on the number of technicians available. A technician's salary was estimated at $7.50 per hr, and no consideration was given to the cost of housing the animals. The final estimate (*65*) was $713.80 for screening one compound.

Norman Klein (1984, personal communication) has estimated that the cost of postimplantation rat embryo culture is $30 per culture bottle, of which $20 is labor cost and the remaining $10 is materials. Since two embryos are cultured per bottle, four treatment groups of 12 embryos per group would require 24 bottles. This would give a minimum cost of $720 for screening one compound.

The initial investment for postimplantation embryo culture is not extraordinary. Major equipment required includes an incubator, dissecting microscopes, rotator apparatus, and either custom gas mixes or a precision three-way gas mixing valve. Provisions for working under sterile conditions are helpful but not absolutely necessary. A suitable laboratory for whole embryo culture could probably be equipped for less than $20,000.

Because all manipulation of the embryos from initial explantation to final evaluation must be done under a microscope, a high degree of skill is required by all personnel. Assuming that enough skilled people were available so that only one experimental run was necessary, 12 days would be required (from mating of rats to completion of culture) to test one agent. This period would be somewhat longer if any elaborate assays were performed on the embryos.

Utility in Mechanistic Studies. Because postimplantation embryos in culture can be observed continuously throughout the period of organogenesis in a well-controlled environment, this system has the potential to provide much useful information about the mechanisms of action of suspect developmental toxicants.

Either parent compounds or known metabolites can be added to the culture medium. This approach provides information on the nature of the proximate teratogen. Embryos can be evaluated biochemically for clues to the underlying mechanisms of teratogenesis. Huber and Brown (*66*) showed that the teratogenic effects of phorbol esters resulted from a disruption of the morphology and function of the visceral yolk sac and not from a direct attack on the embryo. This point is important in relating results of animal experiments to human risks, because human embryos do not have any structure comparable to the rodent yolk sac placenta.

Utility as a Screen for Teratogens. Although whole embryo culture is being actively endorsed as a teratogen screen by several groups (*40,65,67*), only one of these groups (*40*) seems to have taken the necessary steps toward validation. All 18 of the known teratogens tested and 20 of the 21 nonteratogens tested were correctly classified. However, there was no indication that these were blind studies.

Whole embryo culture may also be useful for directly screening humans at reproductive risk and predicting possible palliative measures. Ferrari et al. (*68*) were able to identify nutritional deficiencies in women with poor reproductive histories by the inability of their sera to support development of rat embryos *in vitro*. Dietary supplementation with the appropriate nutrients reversed, in some cases, both the embryotoxicity of the serum and the poor reproductive performance of the woman.

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